Study of biosorption of copper and silver by marine bacterial exopolysaccharides

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Abstract

Metal bioremediation was studied by biosorption of analytical grade copper Cu(II) and silver Ag(I) by an exopolysaccharide (EPS) produced by marine bacteria from French Polynesia. Colorimetric analysis showed that EPS was composed of neutral sugars, uronic acids, acetate and especially high sulfate amount (29%). Metal biosorption experiments were conducted in batch process. Results showed that the maximum sorption capacities calculated according to Langmuir model were 400 mg g^{-1} EPS (6.29 mmol g^{-1}) and 256 mg g^{-1} EPS (2.38 mmol g^{-1}) for Cu(II) and Ag(I), respectively. The influence of pH, biosorbent concentration, ionic strength on EPS biosorption capacities was investigated.

Results showed that bacterial EPS can be considered as very promising for copper and silver bioremediation. Further development in dynamic and continuous process at the industrial scale will be organized next.
Keywords: biosorption, bacterial exopolysaccharides, copper and silver removal, bioremediation.

1   Introduction

Heavy metals are being used widely and largely by industries (e.g. mining, electroplating, welding, electronic…). During the biogeochemical cycles of

metals [1], their speciation and thus their toxicity are modified. That is why, the release into the environment of even trace concentrations of heavy metals can cause severe aquatic or terrestrial damages [2, 3]. In this context and face with more and more stringent regulations, numerous wastewater treatment exist to remove heavy metals from industrial effluents including sorption on activated carbon, precipitation processes, ion exchange, electrochemical methods, membrane systems or chelating resins. Nevertheless, these methods show varying performances, frequently insufficient removal of metal ions, together with high cost and not environmentally friendly [4].

In this situation, biosorption of heavy metals based on metal binding capacities of biological materials can be an alternative solution [5–7]. Among many biosorbents (e.g. bacteria, yeast, seaweed, fungi), bacterial exopolysaccharides (EPS) have been successfully employed in heavy metal removal works [8–11]. The advantage of microbial EPS compared to plants, crustacea or algae is related to their physico-chemical properties and their production by fermentation i.e. that is not subjected to instability due to crop loss, marine pollution or climate change [12]. Moreover, EPS possess ionizable functional groups such as hydroxyl, carboxyl, acetate, phosphate, amine and more rarely sulfate groups, which are possible binding sites for the fixation of heavy metals [13]. It is accepted that metal biosorption involves a physico-chemical interaction between metal cation and functional groups established on ion exchange, physical sorption, complexation and/or precipitation [14]. Furthermore, metal biosorption capacity depends on external parameters such as pH, metal or biomass concentration, ionic strength or nature of counter ions.

Various studies on copper biosorption by EPS have been carried out [11, 15], nevertheless, none have dealt with silver biosorption by purified EPS. Copper and silver are largely used in a broad spectrum of industrial applications (e.g. water purification, mining, electronic…). However, it is well known [3, 13, 15] that both metals present toxicity to aquatic life and constitute serious risk for human health.

The aim of the study was to determine sorption capacity of one bacterial exopolysaccharide for two metals, Cu(II) and Ag(I). Moreover, the influence of initial pH, metal and biosorbent concentration, ionic strength on the binding capacity of these metals by EPS was studied.

2   Materials and methods

2.1 Bacterial strain and EPS production

The strain referenced as M1 was isolated from microbial mats in Rangiroa atoll (French Polynesia) [16, 17]. Bacterial production of exopolysaccharides along with the associated extraction and purification protocols were performed as described previously [17].

2.2 Chemical characterization of EPS

Global composition of EPS M1 (neutral sugars, uronic and hexosamines contents) was characterized using colorimetric methods, while monosaccharide composition and ratios were determined using GC analyses. Non carbohydrate substituents were determined by either HPLC and/or NMR analyses. These chemical EPS analyses were described in previous works [18, 19]. Molecular weight of EPS was established by high-performance size-exclusion chromatography (HPSEC) and polydispersity index (Ip) was calculated from the Mw/Mn ratio (University Le Mans UMR 6120 PCI).

2.3 Chemicals

All chemicals were of analytical grade. Stock solutions of Cu(II) (1000 mg L^{-1}) were prepared by dissolving (Cu(COOCH$_3$)$_2$, Merck) in ultrapure water, and stock solutions of Ag(I) (500 mg L^{-1}) were prepared by dissolving (AgNO$_3$, Sigma Aldrich) in ultrapure water.

2.4 Batch biosorption experiments

In batch biosorption experiments, 50 mg of dry EPS were placed in a flask with 100 mL of metal solution at initial concentrations in the range of 100–1000 mg.L^{-1} for Cu (II) and 50–500 mg.L^{-1} for Ag(I). Silver experiments were carried out in the dark because of silver nitrate photosensibility. Solutions were prepared in triplicate and softly shaken (180 rpm) for 3 hours at 25°C. After agitation, solutions were ultrafiltered using Pellicon® Tangential Flow Filtration Cassettes with a molecular weight cutoff of 100 kDa allowing elimination of free metal ions. Permeates were analyzed to define concentrations of each metal cations while retentates were lyophilized prior to further experiments.

Appropriate controls were analysed during biosorption experiments to control the absence of glassware biosorption of metals and other potential side effects.

To study the effect of pH on biosorption, experiments (50 mg EPS M1.L^{-1} of metal solution) were performed at different pH ranging from 2 to 8. pH was checked at regular intervals and adjusted with 0.1 M NaOH or 0.1 M HCl.

Furthermore, experiments using different EPS concentrations (0.01, 0.05, 0.1, 0.25 and 0.5 g.L^{-1}) were performed to evaluate the optimal silver uptake.

The effect of ionic strength was also studied by using 0.5 g.L^{-1} of EPS to 100 mg.L-1 of Ag(I) or Cu(II) containing different concentration of (i.e. 0.5, 1.0 and 2.0 M) NaCl solutions.

Biosorption capacity q (mg.g^{-1}) at equilibrium per unit mass of EPS was determined using the following equation:

$$q = \frac{(Ci - Ceq).V}{m} \tag{1}$$

where Ci is the initial metal concentration and Ceq the equilibrium metal concentration in solution (mg.L^{-1}) of volume V (mL) and m the mass of EPS (mg).

2.5 Equilibrium biosorption isotherm

Sorption isotherms were plotted according to the linearized model of Langmuir:

$$q = \frac{Q\max.K.[Me]eq}{1+K.[Me]eq} \tag{2}$$

where $Qmax$ (mg.g^{-1}) corresponds to the maximum biosorption capacity, K is the Langmuir equilibrium constant (L.mg^{-1}) and $[Me]eq$ (mg.L^{-1}) the equilibrium concentration of the metal in the solution. Linear transformation of eqn (2) enables the calculation of the Langmuir's parameters.

2.6 Metal analysis

After ultrafiltration, permeates were acidified with HNO_3 (0.5N) and metal concentration was determined by Inductive Coupled Plasma-Atomic Emission Spectroscopy analysis (ICP-AES, HORIBA Jobin YVON ULTIMA 2). ICP analyses were conducted at wavelengths of 324.754 nm, 328.068 nm and 231.604 nm for copper, silver and nickel, respectively. All assays were run in triplicates. All experimental data were presented as mean values ± standard deviations.

3   Results and discussion

3.1 EPS chemical composition

EPS M1 was characterized by percentages of neutral sugars, uronic acids, sulfates and other substituents, Table 1.

Table 1:   Characteristics and chemical composition of EPS M1 [17].

M1 EPS	
Strains	*Paracoccus sp.*
Mw (kDa)	4300
Ip	1.3
Proteins	3
Neutral Sugars	48
Uronic Acids	8
Sulfates	29
Substituents	Acetate

EPS M1 was characterized by a high molecular weight (4300 kDa) and a low polydispersity index (Ip = 1.3) reflecting the homogeneity of the polymer. Chemical composition of EPS M1 is characterized by large amount of neutral sugars (48%), together with 8% uronic acids and high sulphate content reaching 29%, strengthening its anionic character [20, 21]. Furthermore, EPS M1

contained a very low protein amount (<5%) reflecting the efficiency of the purification protocol.

3.2 Metal biosorption of EPS

Biosorption isotherms of Cu and Ag by EPS M1 are shown in Fig. 1. Biosorption isotherms express the equilibrium of metal between the aqueous phase and the EPS versus metal concentration [9, 10]. On this figure, one can clearly observe that biosorption increases with the initial copper and silver concentration.
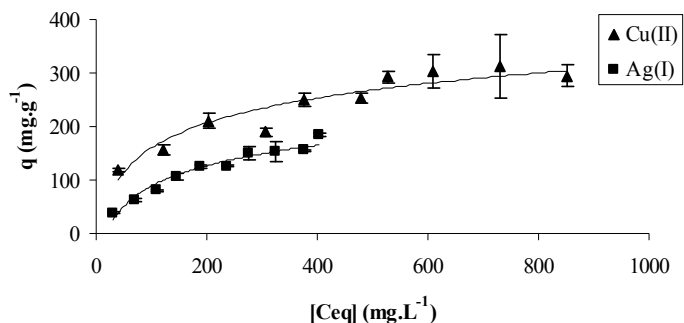

Figure 1:   Equilibrium biosorption isotherms of copper and silver by EPS M1.
Errors bars represent ± standard deviation of triplicate samples.

   Experimental conditions: EPS concentration: 0.5 g.L-1; pH = 6; initial metal concentration: 100–1000 mg.L-1 and 50–500 mg.L-1 for copper and silver, respectively.
   In that, it can be supposed that metal fixation by this EPS is a chemical, equilibrated and saturated mechanism [9, 11]. As shown in Table 2, maximum uptakes (Qmax, calculated from the Langmuir's eqn (2)) were 400 mg Cu.g^{-1} EPS (6.3 mmol Cu.g^{-1} EPS) and 256 mg Ag.g^{-1} EPS (2.38 mmol.g^{-1}). With the exception of a copper removal of 1,602 mg.g^{-1} EPS as observed for *Paenibacillus polymyxa* [22], data from the present work indicated higher value. Furthermore, maximum capacity for silver sorption obtained in this study was much higher than the observed capacities for an EPS slime produced by Alicagenes eutrophus, 79 mg.g^{-1} [23] and other natural biomass reported in the literature ranging between 41.8 and 98.7 mg.g^{-1} [23–25].

Table 2:   Langmuir parameters (Qmax and K) for biosorption of Cu and Ag by EPS M1, and R² determination coefficient.

	Qmax		K		R²
	mg.g^{-1}	mmol.g^{-1}	L.mg^{-1}	L.mmol^{-1}	
Cu	400	6,30	0,00537	341	0,8524
Ag	256	2,38	0,00473	510	0,9484

From these results, it can be hypothesized that EPS composition influences its metal biosorption capacities. In fact, Loaëc *et al.* [10] indicated that the chemical composition of EPS from bacteria *Alteromonas macleodii fijiensis subsp* constituted by neutral sugars, various acids, amino sugars and other components like sulfate and pyruvate esters contributed to the removal of metals. The authors also determined specific functional groups involved in metal removal. Nevertheless, direct link between EPS composition and its biosorption capacity is difficult to establish since biosorption mechanism is probably influenced by numerous other characteristics such as EPS configuration, molecular weight, structure and rheological features.

3.3 Factors influencing biosorption

It is now well accepted that metal sorption implies physicochemical interactions between metallic ions and EPS, and that interactions could be influenced by external factors, such as pH, EPS concentration, and metal concentration [5, 26]. In the present work, EPS M1 was studied to examine influence of varying experimental conditions (pH, initial concentrations, counter ion, ionic strength and heavy metal mixtures) on its metal binding performance for copper and silver.

3.3.1 Effect of pH on biosorption
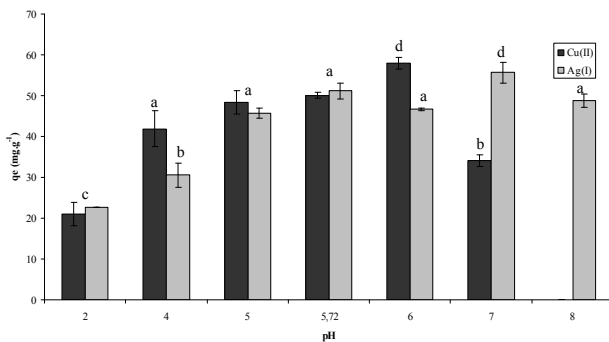The influence of pH on the capacity of biosorption, qe, copper and silver by the EPS M1 is shown in Fig. 2.

Figure 2:   Effect of pH on copper and silver biosorption with EPS M1 (EPS concentration: 0.5 g.L^{-1}). Different letters indicate significant differences according to Tukey's test (p<0.05). Errors bars represent ± standard deviation of triplicate samples.

Biosorption of Cu by EPS M1 was ranged from 21 to 58 mg .g^{-1} for pH values between 2 and 7. At pH = 8, Cu biosorption was zero, then it increased significantly between pH 2 and pH 4, then remained stable between pH 4 and pH = 5.43 to increase again at pH = 6 and finally showed a significant decrease at pH 7 (Fig. 2). Regarding Ag, biosorption varied between 22.6 and 55.6 mg.g^{-1}

for pH between 2 and 8. Then an important increase was observed between pH 2 and pH = 4, followed by quite stable values between pH = 5 and pH = 8 (Fig. 2).

Our results are in agreement with previous works that demonstrated a reduction of metal biosorption with decreasing pH [27, 28]. This phenomenon can be explicated by modification in metal speciation, itself operates on solubility, redox reactions, complexation through metal fixation. [5, 28]. This setting also changes chemical state of biosorbent functional groups. Actually, pH modifies EPS especially by ionization of carboxyl groups (pKa = 4–5) of uronic acids for example. At low pH values, these groups are protonated and therefore available for biosorption of cationic metal. In addition, increase of H_3O^+ ions at low pH values (pH = 2–4) probably compete with metal cations for binding sites and thereby disadvantages metal biosorption by EPS [29]. At pH = 4–5, the competitive effect of protons for binding sites is lower. Moreover, biosorbent negative charge increases until total deprotonation of these functional groups, promoting electrochemical attraction and thus metal biosorption. At pH values above 5, binding capacity of EPS M1 for Ag remained unchanged with a value near 50 mg Ag.g^{-1} EPS.

In the case of copper, beyond pH = 4, biosorption capacity remained stable and then increased again to a maximum pH of 6 (q ~ 60 mg.g^{-1}). For pH = 7, precipitation of copper occurred through the formation of hydroxides and drastically reduced copper biosorption (qe = 34 mg.g^{-1}) (Fig. 2) [30]. This phenomenon was described in many studies of metal biosorption by EPS [10, 27, 31–35].

3.3.2 Effect of EPS concentration on metal biosorption

Results of the effect of concentration on EPS M1 biosorption capacity of Ag and Cu are shown in Fig. 3.
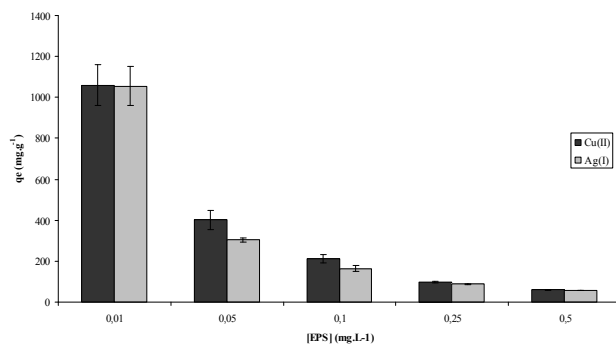

Figure 3:   Effect of EPS M1 concentration on Cu and Ag (50 mg.L^{-1}) biosorption. *Errors bars represent ± standard deviation of triplicate samples.*

At equilibrium, it is noted that the amount of metal sorbed decreases sharply (factor ~20) with increasing EPS concentration (Fig. 3): from 1060 to 60 mg Cu

g^{-1} and from 1050 to 55 mg Ag g^{-1} for EPS M1 concentration ranged from 0.001 to 0.5 g.L^{-1}. According to Ma *et al.* [31] this phenomenon could be attached to the decreasing of metal to EPS ratio. This diminution conduced to more biosorption sites of the EPS remaining unsaturated during the biosorption uptake. Then, high EPS concentration could make a screening effect of the outer layer and thereby shielding the binding sites from metal [36].

3.3.3 Effect of ionic strength

Industrial effluents often contain large amounts of alkali or alkaline earth metals such as Ca^{2+}, Na^+, Mg^{2+}. In this context, NaCl solution at various concentrations (0.5, 1.0 and 2.0 M) was added to the EPS-metals solution to study the effect of ionic strength on biosorption. Results are presented in Fig. 4.
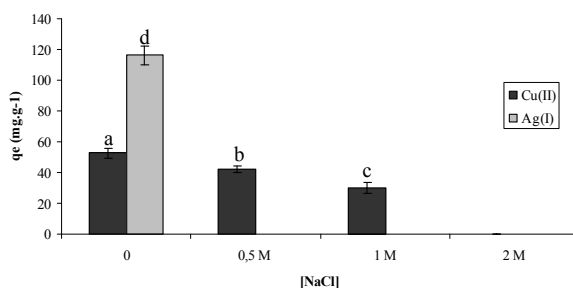

Figure 4:   Effect of ionic strength (NaCl) on the biosorption of Cu and Ag (100 mg.L-1) by EPS M1. Different letters indicate significant differences according to Tukey's test (p<0.05). Errors bars represent ± standard deviation of triplicate samples.

It can be observed a significant decrease of EPS M1 biosorption capacity of copper with increasing ionic strength, with values of qe falling from 53 to 30 mg.g^{-1} after increasing NaCl concentration from 0 to 1.0 M (Fig. 4). With regards to silver biosorption, introducing of 0.5 M NaCl, decreased biosorption capacity to zero. This can be again explained by the formation of chlorocomplexes as previously discussed. Concomitant decrease of biosorption with progressive ionic strength concentration can be explained by a competition between metal cations and Na^+ ions for binding sites, and/or a possible masking of binding sites by sodium ions as it was observed elsewhere [37].

4   Conclusion

In this study, we demonstrated the binding capacities of marine bacterial EPS M1 for Ag^+ and Cu^{2+} heavy metals. Maximum uptake capacities as high as 400 mg of Cu(II)/g and 256 mg of Ag(I)/g were determined for EPS M1. Factors such as pH, EPS concentration and ionic strength highly influenced biosorption of Ag(I) and Cu(II). These results can be partly related to the specific chemical composition of EPS M1 and the number of available binding sites. These results

showed that this bacterial EPS can be considered as very promising candidate for bioremediation of silver and copper. With the view of industrial development of EPS based metal remediation, studies dealing with immobilization of EPS onto regenerative matrix and further desorption of metal are planned in our laboratory. Further development in dynamic and continuous process at the industrial degree will be installed next.

Acknowledgements

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